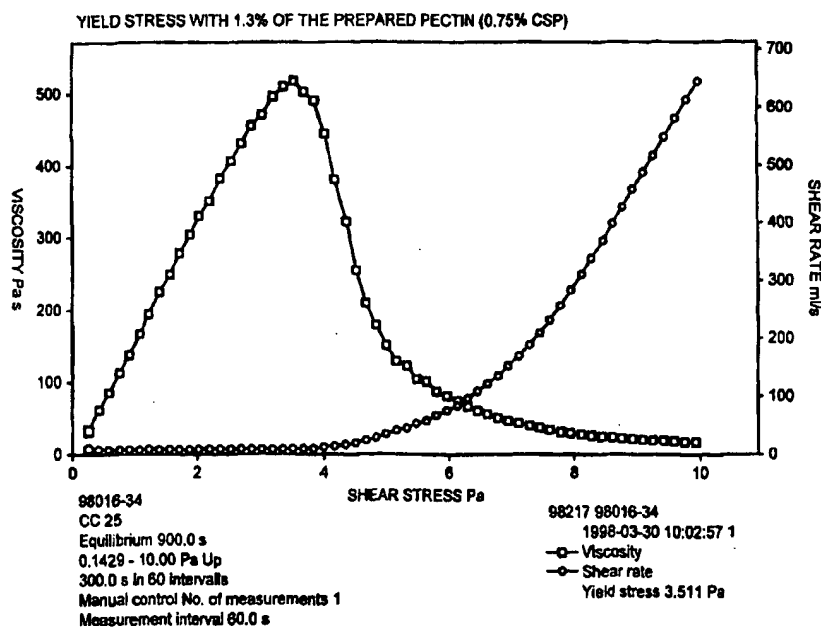




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : A23L 1/0524, 1/064, 2/52, A23C 9/123, A23L 1/24, A23G 9/00		A1	(11) International Publication Number: <b>WO 00/08952</b> (43) International Publication Date: 24 February 2000 (24.02.00)
(21) International Application Number: PCT/IB99/01476 (22) International Filing Date: 13 August 1999 (13.08.99) (30) Priority Data: 9817805.6 14 August 1998 (14.08.98) GB (71) Applicant (for all designated States except US): DANISCO A/S [DK/DK]; Langebrogade 1, P.O. Box 17, DK-1001 Copenhagen K (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): MADSEN, Finn [DK/DK]; Soskraenten 7, DK-8260 Viby J (DK). GROVEN, Stig [DK/DK]; Oddervej 70, DK-8700 Horsens (DK). (74) Agents: MASCHIO, Antonio et al.; D. Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published With international search report.	

(54) Title: USE OF A COMPOSITION



## (57) Abstract

There is provided use of a pectin composition to prepare an aqueous composition having high yield stress characteristics without substantial gelation, wherein the pectin composition comprises at least a population of pectin that is calcium sensitive; wherein the pectin composition is capable of imparting high yield stress characteristics to an aqueous composition without causing substantial gelation of the aqueous composition.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LJ	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

### Use of a Composition

The present invention relates to a use of a composition. In particular, the present invention relates to a use of a pectin composition.

5

Pectin is an important commodity in today's industry. For example, it can be used in the food industry as a thickening or gelling agent, such as in the preparation of jams or fruit systems for yoghurt.

10

Pectin is a structural polysaccharide commonly found in the form of protopectin in plant cell walls. The backbone of pectin comprises  $\alpha$ -1-4 linked galacturonic acid residues which are interrupted with a small number of 1,2 linked  $\alpha$ -L-rhamnose units. In addition, pectin comprises highly branched regions with an almost alternating rhamno-galacturonan chain. These highly branched regions also contain other sugar units (such as D-galactose, L-arabinose and xylose) attached by glycosidic linkages to the C3 or C4 atoms of the rhamnose units or the C2 or C3 atoms of the galacturonic acid units. The long chains of  $\alpha$ -1-4 linked galacturonic acid residues are commonly referred to as "smooth" regions, whereas the highly branched regions are commonly referred to as the "hairy regions".

20

Some of the carboxyl groups of the galacturonic residues are esterified (e.g. the carboxyl groups are methylated). Typically esterification of the carboxyl groups occurs after polymerisation of the galacturonic acid residues. However, it is extremely rare for all of the carboxyl groups to be esterified (e.g. methylated). Usually, the degree of esterification will vary from 0-90%. If more than 50% of the carboxyl groups are esterified then the resultant pectin is referred to as a "high ester pectin" ("HE pectin" for short) or a "high methoxyl pectin". If less than 50% of the carboxyl groups are esterified then the resultant pectin is referred to as a "low ester pectin" ("LE pectin" for short) or a "low methoxyl pectin". If 50% of the carboxyl groups are esterified then the resultant pectin is referred to as a "medium ester pectin" ("ME pectin" for short) or a "medium methoxyl pectin". If the pectin does not contain any - or only a few - esterified groups it is usually referred to as pectic acid.

30

SUBSTITUTE SHEET (RULE 26)

LE pectins have for a long time been used in yoghurt fruit as a stabilising agent to ensure a certain yield stress and consequently uniform distribution of fruit without giving a too high viscosity. However, LE pectins tend to form a gel and any gelation should be avoided.

Gelation is detrimental to the appearance of many food products and may also result in undesirable organoleptic properties. Gelation of a product may also prohibit the pumping of that product. Pumping is a widely used method for manipulating products during manufacture and application, and consequently pumpability is a demand in many applications, e.g. yoghurt fruit application.

The prior art has attempted to overcome the above problems. The prior art has provided "pregelled" systems which although they may overcome some of the above problems, produce a product which is grainy with a dull appearance. Furthermore, such pregelled products has a strong tendency to syneresis.

The present invention aims to overcome the problems of the prior art.

According to a first aspect of the present invention there is provided use of a pectin composition to prepare an aqueous composition having high yield stress characteristics without substantial gelation, wherein the pectin composition comprises at least a population of pectin that is calcium sensitive; wherein the pectin composition is capable of imparting high yield stress characteristics to an aqueous composition without causing substantial gelation of the aqueous composition.

The present invention is advantageous in that it may provide an aqueous composition which is smooth and shiny in appearance without exhibiting syneresis, in contrast to the pre-gelled systems of the prior art.

The term "pectin" includes fractions of pectin, one or more compounds from the class of compounds known as pectins, and derivatives thereof.

SUBSTITUTE SHEET (RULE 26)

The term "derivatives thereof" includes derivatised pectin and degraded pectin (such as partially degraded pectin) and modified pectin.

5 The term "population of pectin that is calcium sensitive" means a population of pectin which has a calcium sensitivity index (CF) not equal to 1. A Protocol for determining calcium sensitivity may be found on page 57 of WO-A-97/03574 (the contents of which are incorporated herein by reference). For ease of reference, this Protocol is recited at the end of the Examples section (*infra*) as Protocol I.

10 Preferably, the population of pectin has a CF of greater than 1.2. More preferably, the population of pectin has a CF of greater than 5. Yet more preferably, the population of pectin has a CF of greater than 15.

15 Preferably, the pectin composition has a CF of greater than 1.2. More preferably, the pectin composition has a CF of greater than 5. Yet more preferably, the pectin composition has a CF of greater than 15.

20 In the present specification, by the term "having a high yield stress" it is meant that the yield stress of the aqueous composition as measured in accordance with the Protocol II is at least 1.0 Pa. Protocol II is recited at the end of the Examples section (*infra*)

25 Preferably, the yield stress of the aqueous composition is greater than 3.0 Pa. Yet more preferably, the yield stress of the aqueous composition is greater above 5.0 Pa.

30 In the present specification, by the term "without substantial gelation" it is meant that the maximum viscosity of a standardised aqueous composition measured in accordance with the Protocol III is no greater than 500 Pa. Protocol III is recited at the end of the Examples section (*infra*).

Preferably, the pectin composition is capable of imparting a high degree of shear

SUBSTITUTE SHEET (RULE 26)

thinning to an aqueous composition. A high degree of shear thinning is an important rheological characteristic in liquid and semi-liquid systems to facilitate pumping and to secure a good non-sticky mouthfeel.

5 The degree of thixotropy of the aqueous composition may be varied from application to application. If stabilisation of suspended particles is required, the aqueous composition is preferably able to build up structure after shearing with low thixotropy. If a degree of flow after shearing is required, a degree of thixotropy is desirable and may be provided by the pectin composition. The pectin composition  
10 may be used in accordance with the present invention to control the above rheological characteristics. This control may be achieved by controlling, among other things, the degree of esterification (%DE), the proportion of the pectin composition which is calcium sensitive (%CSP), the calcium sensitivity of the calcium sensitive population of pectin (CS), the molecular weight of the pectin  
15 composition and/or the concentration of calcium in the aqueous composition.

Degree of thixotropy, shear thinning and shear reversibility may be evaluated by performing a flow curve experiment in accordance with Protocol IV. Protocol IV is recited at the end of the Examples section (*infra*).

20

Preferably, the aqueous composition has a low viscosity at a shear rate of  $0.1 \text{ s}^{-1}$ .

25

In the present specification, by the term "low viscosity" it is meant that the viscosity at a shear rate of  $0.1 \text{ s}^{-1}$ , measured in accordance with Protocol IV, is less than  $100 \text{ Pa.s}$ , preferably less than  $80 \text{ Pa.s}$ .

30

Thus, in a second aspect the present invention provides use of a pectin composition to prepare an aqueous composition having high yield stress characteristics and a low viscosity at a shear rate of  $0.1 \text{ s}^{-1}$ , wherein the pectin composition comprises at least a population of pectin that is calcium sensitive; wherein the pectin composition is capable of imparting to an aqueous composition high yield stress characteristics and a low viscosity at a shear rate of  $0.1 \text{ s}^{-1}$ .

SUBSTITUTE SHEET (RULE 26)

The calcium sensitive pectin population can comprise any one or more of a low ester pectin, a medium ester pectin or a high ester pectin. Preferably, the calcium sensitive pectin population comprises a high ester pectin. Preferably, the calcium sensitive pectin population does not comprises a low ester pectin or a medium ester pectin.

Preferably, the degree of esterification of the calcium sensitive pectin population is from 50 to 90%. More preferably, the degree of esterification is from 60 to 85%. More preferably, the degree of esterification is from 60 to 80% or 65 to 85%. Yet more preferably, the degree of esterification is from 65 to 80%.

Preferably, the degree of esterification of the total pectin composition is from 50 to 90%. More preferably, the degree of esterification is from 60 to 80% or 65 to 85%. Yet more preferably, the degree of esterification is from 65 to 80%.

A Protocol for determining the degree of esterification of a pectin may be found on page 58 of WO-A-97/03574 (the contents of which are incorporated herein by reference). For ease of reference, this Protocol is recited at the end of the Examples section (*infra*) as Protocol V.

The structure of pectin, in particular the degree of esterification of pectin and the degree of block structure i.e. the degree of distribution of ester groups and free carboxyl groups in blocks, controls many of the physical properties of the pectin. Thus, modification and/or control of the degree of esterification and degree of block structure may be important.

The degree of esterification and/or degree of block structure may be modified and/or controlled by any method. Examples of suitable methods are acid hydrolysis, alkali hydrolysis, use of other chemical de-esterification agents, or by use of enzymes. Suitable enzymes can be obtained from plants or micro-organisms such as bacteria, yeast or fungi. The enzymes disclosed in the review prepared by Pilnik and Voragen (Food Enzymology, Ed.: P.F.Fox; Elsevier; (1991); pp: 303-337) may be used.

SUBSTITUTE SHEET (RULE 26)

Preferably, the degree of esterification of the pectin composition is modified and/or controlled by use of a pectin methylesterase (EC 3.1.1.11), otherwise referred to as a PME. PMEs de-esterify HE pectins to LE pectins or pectic acids.

5

The use of PMEs is advantageous because PME activity produces free carboxyl groups and free methanol. The increase in free carboxyl groups and thus the degree of esterification can be easily monitored by automatic titration.

10

For example, the degree of esterification of the calcium sensitive pectin population is modified and/or controlled in accordance with a process described in WO 98/47391 (which claims priority from British Patent Application No 9708278.8).

15

Preferably the pectin composition is obtainable or is obtained by fractionation of a pectin or a pectin composition. Using fractionation techniques a pectin composition may be obtained which is enriched in one or more specific calcium sensitive pectin populations.

20

Preferably the pectin composition is obtainable or is obtained by selecting peels from a citrus fruit, for instance lime and/or lemon, and extracting the pectin by a process comprising the steps of:

#### Extraction

Mixing the peel with water at a water:peel ratio of 21:1

25

Adjusting, if necessary, the pH to 1.9-2.3 using nitric acid

Heating to a temperature of 68-71° C for 3-6 hours

#### Filtration

After extraction the pectin solution is filtered.

30

#### Precipitation

Reducing the temperature to 36-40 °C

SUBSTITUTE SHEET (RULE 26)



Adjusting the pH to 2.6-2.9

Adding isopropyl alcohol until precipitation occurs. Typically an isopropyl alcohol concentration of 49-58 w/w % is required.

5 Post treatments

After precipitation the pectin may be dried and/or milled.

Preferably, the use of the present invention provides an aqueous composition.

10 Thus, in a third aspect of the present invention there is provided an aqueous composition prepared in accordance with the use of the present invention.

The aqueous composition of the present invention may comprise one or more other components, such as one or more suitable food ingredients. Typical food ingredients  
15 include any one or more of a salt, a protein, an acid - such as citric acid - or a sugar - such as sucrose, glucose or invert sugar - or fruit - or enzymes.

The present invention can be used in the preparation of pharmaceutical products, foodstuffs and non-food products.

20

Typical pharmaceutical products include clinical nutritive products (enteral products and other liquid products).

Typical non-food products include de-icing agents and paints.

25

The term "foodstuff" can include food for human and/or animal consumption.

30

Typical foodstuffs include jams, fruit preparations, fruit fillings, ripples, fruit sauces, stewed fruit, dairy products (such as milk products and ice cream), fine foods (such as salad dressings, ketchup, vinaigrette dressings and soups), meat products, poultry products, fish products and bakery products. The foodstuff may be a beverage. The beverage may be a drinking yoghurt, a fruit juice, a beverage concentrate or a fruit

SUBSTITUTE SHEET (RULE 26)

based beverage.

The present invention may be used in the preparation of a starting reagent or an intermediate in the preparation of a foodstuff.

5

Alternatively, present invention may be used in the preparation of a foodstuff itself.

10

Preferably, the pectin composition comprises no greater than 5.0 wt % of aqueous composition based on the total weight of the aqueous composition. More preferably, the pectin composition comprises no greater than 0.1-1.5 wt % of aqueous composition based on the total weight of the aqueous composition.

15

Preferably, the pectin composition and/or the aqueous composition further comprises one or more metal ions. Preferably, the one or more metal ions comprise metal ions selected from ions of elements of Group II of the Periodic Table. Yet more preferably, the one or more metal ions comprise at least  $\text{Ca}^{2+}$  ions.

20

In a further preferred embodiment, the pectin composition and/or aqueous composition comprises 25-300 mg of metal ions per gram of pectin. In yet a further preferred embodiment, the pectin composition and/or aqueous composition comprises 50-150 mg of metal ions per gram of pectin.

25

In a further preferred embodiment, the pectin composition and/or aqueous composition comprises 25-300 mg of  $\text{Ca}^{2+}$  ions per gram of pectin. In yet a further preferred embodiment, the pectin composition and/or aqueous composition comprises 50-150 mg of  $\text{Ca}^{2+}$  ions per gram of pectin.

30

In the above embodiment the metal ions may be introduced in to the pectin composition by adding the metal ions to the pectin composition during production thereof or it can be added to the dry pectin composition afterwards. In one alternative, the metal ions may be introduced in to the pectin composition by adding the metal ions to the pectin composition in the presence of water.

SUBSTITUTE SHEET (RULE 26)

The metal ions may be introduced in to the aqueous composition by adding the metal ions directly thereto before or after the introduction of the pectin composition in to the aqueous composition.

5

The metal ions, preferably calcium, may be added as a metal ion salt. For example the metal ion may be added in the citrate form of the metal, for example, calcium citrate.

10

An example of the combination of metal ions and pectin composition in accordance with the present invention to provide an aqueous composition in accordance is given below:

15

- 1) A pectin with a pH value of 5 is mixed with calcium citrate
- 2) The mixture is dissolved in water. Under the pH conditions i.e. a pH of 5, only the pectin will dissolve.
- 3) The pH is lowered to a level below 4. On lowering pH the calcium citrate will dissolve, releasing the calcium. Once the calcium is released it will become available and an aqueous composition exhibiting high yield stress characteristics without substantial gelation is obtained.

20

It is essential that the pectin composition for use in the present invention comprises a population of pectin that is calcium sensitive. However, the pectin composition for use in the present invention may further comprise at least one calcium insensitive pectin.

25

Preferably, the population of pectin that is calcium sensitive comprises at least 40 wt% of the pectin composition. More preferably, the population of pectin that is calcium sensitive comprises at least 50 wt% of the pectin composition. More preferably, the population of pectin that is calcium sensitive comprises at least 60 wt% of the pectin composition. More preferably, the population of pectin that is calcium sensitive comprises at least 70 wt% of the pectin composition. Yet more preferably, the population of pectin that is calcium sensitive comprises at least 80 wt% of the pectin composition.

30

SUBSTITUTE SHEET (RULE 26)

The proportion of the pectin composition that is calcium sensitive may be determined in accordance with the method of Protocol VI. . Protocol VI is recited at the end of the Examples section (*infra*).

5

Preferably, the pectin composition has a reduced viscosity of at least 0.25 L/g. More preferably, the pectin composition has a reduced viscosity of at least 0.375 L/g. Yet more preferably, the pectin composition has a reduced viscosity of at least 0.50 L/g. Reduced viscosity may be measured in accordance with Protocol VII. Protocol VII is recited at the end of the Examples section (*infra*).

10

In the present specification we have described that the present invention relates to the use of a pectin composition. However, the present invention also encompasses the use of any polysaccharide to provide the advantageous properties of the present invention. Thus, in a broad aspect, the present invention provides the use of a polysaccharide composition to prepare an aqueous composition having high yield stress characteristics without substantial gelation; wherein the polysaccharide composition is capable of imparting high yield stress characteristics to an aqueous composition without causing substantial gelation of the aqueous composition.

15

20

Alternatively, in a broad aspect the present invention provides the use of a composition comprising a pectin isostere to prepare an aqueous composition having high yield stress characteristics without substantial gelation, wherein the pectin isostere composition is capable of imparting high yield stress characteristics to an aqueous composition without causing substantial gelation of the aqueous composition.

25

In a further broad aspect the present invention provides a pectin composition, wherein the pectin composition comprises at least a population of pectin that is calcium sensitive; wherein the pectin composition is capable of imparting high yield stress characteristics to an aqueous environment without causing substantial gelation of the aqueous environment. In this broad aspect the present invention further

30

SUBSTITUTE SHEET (RULE 26)

11

provides a process for preparing an aqueous composition, preferably a foodstuff, comprising the step of combining an aqueous medium with a pectin composition, wherein the pectin composition comprises at least a population of pectin that is calcium sensitive; wherein the pectin composition is capable of imparting high yield stress characteristics to an aqueous environment without causing substantial gelation of the aqueous environment.

The invention will now be described, by way of example only, with reference to the accompanying drawings in which:-

Figure 1 is a graph of the analysis of a composition.

Figure 2 is a graph of the analysis of a composition.

Figure 3 is a graph of the analysis of a composition.

Figure 4 is a graph of the analysis of a composition.

Figure 5 is a graph of the analysis of a composition.

Figure 6 is a graph of the analysis of a composition.

Figure 7 is a graph of the analysis of a composition.

Figure 1 shows a yield stress measurement of a pectin composition for use in accordance with the present invention. The procedure for measuring yield stress is described in Protocol II. The yield stress is determined as the force needed (Pa) to break down the structure (point of maximum viscosity). It is seen that the yield stress is rather high, and the viscosity is low (500 Pa s) at structure breakage. It is furthermore seen that the viscosity curve is bell shaped in nature meaning that the breakdown of structure only gradually takes place at stress levels higher than the yield stress. This is a characteristic property of pectin compositions which may be used in accordance with the present invention. The property means in practice that the pectin composition, even at higher stress levels than the yield stress, retains a substantial part of its structure and thereby stabilising ability. In quantitative terms this is seen as a rather high viscosity and low shear rate measured at stresses somewhat above the yield stress.

SUBSTITUTE SHEET (RULE 26)

In a preferred aspect the pectin composition of the present invention has a shear rate of no greater than 500 ml/s at a stress value 2 Pa higher than the yield stress when measured in accordance with Protocol II. This behaviour is completely different from a soft gelled and brittle texture typically obtained by gelling hydrocolloids, where the viscosity curve after passing the yield stress point shows fast reduction of viscosity and the shear rate is increased strongly, illustrating complete loss of stabilising ability. Visually such products are evaluated as brittle gels. Figure 1 may be compared with figure 2 which shows a yield stress measurement of an aqueous composition of a low ester amidated pectin, where a pregelled texture is obtained to avoid gelling. Such an aqueous composition of a low ester amidated pectin composition would typically be used in food applications. Even though a bell shaped yield stress curve is obtained indicating stabilising ability even somewhat above the yield stress, the aqueous composition of the low ester amidated pectin composition is disadvantageous due to the very high viscosity at the yield stress. Moreover the aqueous composition of low ester amidated pectin composition is disadvantageous, because it is dull and grainy.

When Figure 1 is compared to Figure 3 it can be seen that the yield stress level, bell shaped viscosity curve and viscosity at structure breakage of the pectin composition for use in the present invention are comparable with that obtained when using a 0.7 % xanthan composition. Figure 1 may be compared in a similar manner to Figure 7 illustrating the yield stress measurement of a 0.8 % xanthan composition.

Thus the pectin composition for use in the present invention provides an aqueous composition having high yield stress and still a low viscosity, and furthermore the appearance of the aqueous composition is smooth and shiny, in contrast to the product obtained using low ester amidated pectin.

Fig. 4 is a graph of the viscosity at varying shear rates ( "flow curves") of a yoghurt fruit recipe prepared in accordance with the present invention. The procedure for measuring flow curves is described in protocol IV. For comparison, flow curves are also produced on the same yoghurt fruit recipe, where the pectin composition has been substituted with 0.55 % low ester amidated pectin or 0.7 % xanthan. It can be seen,

that the viscosity at low shear rates (e.g.  $0.1 \text{ s}^{-1}$ ) for the aqueous composition comprising pectin composition used in the present invention is lower than for the aqueous composition comprising low ester amidated pectin and more comparable with xanthan.

Two aqueous compositions, one containing a pectin composition consisting of calcium sensitive pectin population and the other containing a pectin composition consisting of a non-calcium sensitive pectin population were produced to document the importance of the presence of a calcium sensitive pectin population. A high ester pectin raw material was fractionated into two pectin compositions. One of the pectin compositions (referred to as CSP composition) contained almost pure calcium sensitive pectin (CSP), determined in accordance with the method of Protocol VI. The other pectin composition (referred to as NCSP composition) contained 0% CSP (100% non calcium sensitive pectin (NCSP)). The CSP composition and NCSP composition were tested for yield stress properties in accordance with Protocol II. A fixed dosage of 0.75% CSP and 0.75% NCSP were used, respectively.

Figures 5 and 6 shows the yield stress values obtained. These figures demonstrate that the presence of a calcium sensitive pectin population is essential in the pectin composition for use in the present invention.

The preparation of a pectin in accordance with the present invention and its use in a composition in accordance with the present invention will now be described.

## EXAMPLES

### Preparation of Pectin

A pectin composition for use in accordance with the present invention may be obtained by selecting peels from a citrus fruit, for instance lime and/or lemon, and extracting the pectin composition by a process comprising the steps of:

14

Extraction

Mixing the peel with water at a water:peel ratio of 21:1

Adjusting, if necessary, the pH to 1.9-2.3 using nitric acid

Heating to a temperature of 68-71° C for 3-6 hours

5

Filtration

After extraction the pectin solution is filtered.

Precipitation

10

Reducing the temperature to 36-40 °C

Adjusting the pH to 2.6-2.9

Adding isopropyl alcohol until precipitation occurs. Typically an isopropyl alcohol concentration of 49-58 w/w % is required.

15

Post treatments

After precipitation the pectin may be dried and/or milled.

The pectin composition obtained by this process was analysed to have the following characteristics:

20

Degree of esterification (%DE)	68.1 %
Loss on drying	10.6 %
pH	3.1
Calcium sensitivity (CF)	10.4
CSP content	59.1 %

25

Example 1 - Fruit Preparation with 30% Soluble Solids

A fruit preparation having a formulation shown in Table I below was prepared in accordance with the following procedure.

30

1) Dry blend the pectin, and sugar and dissolve the blend in hot water (80-90°C), while

SUBSTITUTE SHEET (RULE 26)



15

agitating vigorously.

2) Mix fruit, sugar and heat to 80-90°C.

3) Add 1) to 2), while agitating vigorously.

4) Heat for some time and add additional water to adjust the solid content.

5) Add calcium lactate (preferably as a slurry) at a minimum temperature of 90°C under agitation

6) Adjust pH to 3.8-4.0, adjust the soluble solid content to 30 % and add flavouring.

7) Cool to 30°C under agitation

8) Fill at 30°C.

10

The fruit preparation made according to Example 1 gives a yield stress around 3 Pa which secures very good fruit piece suspension. At the same time the fruit preparation has a low viscosity compared to similar formulations made with traditional amidated low ester pectin giving a fruit preparation with good flowability and pumpability and a very clean mouthfeel property. These properties make the fruit preparation useful in many types of yoghurts such as stirred fruit yoghurt, layered yoghurts and in twinpot systems as mentioned below.

15

A stirred fruit yoghurt may be prepared by mixing the fruit preparation into yoghurt (white base), for example, in an amount of 10-20 wt% based on the yoghurt.

20

The fruit preparation may also be used in two or multi-layered yoghurts without the formation of a gelled layer at the interface.

25

The fruit preparation may also be used for twin pot systems (where the fruit preparation is in a separate section to the white base yoghurt) giving a fruit preparation with complete fruit piece suspension and a shiny and smooth appearance.

The fruit preparation may also be used for various dessert products.

SUBSTITUTE SHEET (RULE 26)

**TABLE I**

Ingredients	wt %
Pectin composition according to the invention	1.3*
Sugar	1.8
Water	24.7
Strawberry, sliced	50.0
Sugar	20.4
Calcium lactate, 5H <sub>2</sub> O	1.5
Strawberry Flavouring 10565, NID**	0.3
Total	100.0
Final pH	3.8-4.0
Final % soluble solids	30
Filling Temperature	30°C

\* corresponding to a calcium sensitive pectin population content of 0.75 wt%

5 \*\* NID - Nature Identical

#### Example 2 - Fruit Preparation with 50% Soluble Solids

10 A fruit preparation having a formulation shown in Table II below was prepared in accordance with the procedure used in Example 1. The fruit preparation was incorporated into a yoghurt as described in Example 1.

The fruit preparation has a high yield stress and good fruit piece suspending properties. Also this fruit preparation has a considerably lower viscosity compared to similar  
15 formulations made with traditional amidated low ester pectins, resulting in good flowability and pumpability, and a very clean mouthfeel property.

As mentioned the fruit preparation may be used in all types of yoghurts such as stirred

SUBSTITUTE SHEET (RULE 26)

fruit yoghurt, layered yoghurts and in twinpot systems, but also in other dessert systems.

**TABLE II**

5

Ingredients	wt %
Pectin composition according to the invention	1.3*
Sugar	1.4
Water	7.0
Strawberry, sliced	45.0
Sugar	41.3
Water	2.2
Calcium lactate, 5H <sub>2</sub> O	1.5
Strawberry Flavouring 10565, NID**	0.30
Total	100.0
Final pH	3.8-4.0
Final % soluble solids	50
Filling Temperature	30°C

\* corresponding to a calcium sensitive pectin population content of 0.75 wt%

\*\* NID - Nature Identical

10 **Example 3 - 10% Fruit Drink**

A fruit drink having a formulation shown in Table III below was prepared in accordance with the following procedure.

- 15
- 1) Dry blend pectin with sugar
  - 2) Dissolve dry-blend in water at 80°C by agitating vigorously for 15 minutes
  - 3) Add fruit juice, concentrate or puree and add any remaining water

SUBSTITUTE SHEET (RULE 26)

18

- 4) Adjust pH and add flavourings and preservatives
- 5) Add calcium lactate (preferably as a slurry)
- 6) Heat treat the beverage at 80-90°C either in a continuous or in a batch system
- 7) De-aerate the beverage, if necessary
- 5 8) Cool to filling temperature and fill aseptically

The viscosity level obtained in the fruit drink is relatively low - around 25 mPas (at a shear rate/second of 50) and is comparable to a similar fruit drink made with a CMC and Xanthan gum stabiliser system (GRINDSTED™ JU 543 Stabiliser System  
 10 available from Danisco Ingredients, Denmark) used in a 0.2% dosage.

Also the yield stress and thus the pulp stabilising effect of the pectin based sample is comparable with the CMC and Xanthan gum based reference (0.2% dosage).

15 **TABLE III**

Ingredients	wt %
Pectin composition according to the invention	0.40*
Orange concentrate	2.0
Sugar	11.50
Flavouring	0.3
Water	85.3
Calcium lactate, 5H <sub>2</sub> O	0.5
Total	100.0

\* corresponding to a calcium sensitive pectin population content of 0.25 wt%

#### Example 4 - Fruit Drink Concentrate

20 Cordial/Squash (Dilution ratio 1:9)

A dilutable fruit drink concentrate having a formulation shown in Table IV below was prepared in accordance with the following procedure.

SUBSTITUTE SHEET (RULE 26)

- 1) Dry blend pectin with sugar
- 2) Add dry-blend to water phase
- 3) Heat to 90°C for a few minutes, while agitating
- 5 4) Add the juice concentrate and continue the heating a few minutes
- 5) Add the calcium lactate (preferably as a slurry)
- 6) Dissolve the rest of the sugar at 80°C
- 7) De-aerate under vacuum at 80°C
- a) Cool to 40°C or below and add flavourings, preservatives and clouding agent
- 10 9) Continue agitating until the product is homogeneous
- 10) Bottle/fill

Before consumption, the concentrate has to be diluted with tap water in a 1:9 ratio by the consumer. Thus, 1 litre of concentrate gave 10 litres of a ready-to-drink beverage. The beverage is a fresh and slightly acidic fruit drink.

The texture obtained in the beverage concentrate made according to the present invention using the described pectin is comparable with traditional cordial/squash based on alginate/alginic acid as thickener and stabilising agent, used in an equivalent dosage. Thus the ability of the pectin based fruit drink concentrate to be diluted with water is comparable with that of the reference sample.

The use of pectin based fruit drink concentrates will also be applicable in sugar reduced or sugar free formulations. In such formulations the reference will often be stabilised with xanthan gum based stabiliser systems.

**TABLE IV**

Ingredients	wt %
Pectin composition according to the invention	0.3*
Sugar	3.0

SUBSTITUTE SHEET (RULE 26)

	20
Water	31.85
Juice concentrate 65-67°Brix	15.0
Calcium lactate, 5H <sub>2</sub> O	0.4
Sugar	47.96
Preservative	(as needed)
Clouding agent (optional)	1.0
Flavourings	0.5
Total	100.0
Final pH	3.5

\* corresponding to a calcium sensitive pectin population content of 0.2 wt%

5 If the °Brix is lower for the juice concentrate added, the formulation has to be modified to obtain the right final soluble solid content.

#### Example 5 - Vinaigrette Dressing

10 A vinaigrette dressing having a formulation shown in Table V below was prepared in accordance with the following procedure.

- 1) Mix water and pectin and heat to 85°C
- 2) When pectin is dissolved cool to 30°C
- 3) Add salt, sugar and spices and herb to the solution
- 15 4) Add oil phase to the solution
- 5) Add vinegar to the solution
- 6) Mix for approximately 15 minutes
- 7) Add calcium lactate slurry
- 8) Fill

20

The formulation in table V was compared with a similar formulation based on Xanthan gum in 1:1 substitution.

SUBSTITUTE SHEET (RULE 26)

The dressings obtained have the same stability and appearance in an organoleptic evaluation. However the dressing made with the pectin has a yield stress of around 1.5 Pa compared to the xanthan based sample which has a yield stress of more than 4 Pa.

- 5 The organoleptic evaluation also shows that the pectin based sample has a very characteristic mouth-feel property in terms of less sticky texture, a more clean eating property and a better flavour release compared to the xanthan based sample.

10 Viscosity measurements shows that the viscosity of the two samples is close to each other (measured at a shear rate/sec between 50 and 100).

TABLE V

Ingredients	wt %
Water	48.6
Sugar	9.00
Vinegar 10%	18.00
Salt	1.00
Soya oil	22.00
Parsley flakes	0.03
Black pepper granulate	0.02
Mix of herbs	0.05
Calcium lactate, 5H <sub>2</sub> O	0.65
Pectin composition according to the invention	0.65*
Total	100.00

- 15 \* corresponding to a calcium sensitive pectin population content of 0.40 wt%

Example 6 - Dressing

A dressing having a formulation shown in Table VI below was prepared in

SUBSTITUTE SHEET (RULE 26)

accordance with the following procedure.

- 1) Blend water, sugar, potassium sorbate and salt
- 2) Blend pectin, propylene glycol alginate (PGA) and starch with a little oil and add  
5 to the water phase
- 3) Mix until all ingredients are fully dissolved
- 4) Add egg yolk to the water phase
- 5) Add calcium lactate to the water phase
- 6) Emulsify the remaining of the oil into the water phase
- 10 7) Add vinegar and mustard
- 8) Fill

The dressing produced has a good body and cling, and the same texture and stability as  
15 typical commercial formulations.

**TABLE VI**

Ingredients	wt %
Water	50.50
Soya oil	30.00
Salt	1.50
Sugar	4.50
Ultratex 4	2.00
Propylene glycol alginate (PGA)	0.20
Calcium lactate, 5H <sub>2</sub> O	0.60
Pectin composition according to the invention	0.5*
Fluid egg yolk	4.00
Vinegar 12 %	5.00
Mustard	1.00
Potassium sorbate	0.20
Total	100.00

SUBSTITUTE SHEET (RULE 26)



\* corresponding to a calcium sensitive pectin population content of 0.4 wt%

Example 7 - Ice cream

Ice cream having the formulation shown in Table VII below was prepared according to the following procedure:

- 1) Heat all liquid ingredients to approx. 40°C
- 2) Mix pectin, emulsifier and sugar
- 3) Mix the dry blend with the liquid ingredients
- 4) Pasteurise at 80-85°C for 20-40 seconds
- 5) Homogenise at 80°C
- 6) Cool to ageing temperature, 4°C
- 7) Age for min. 4 hours (preferably overnight)
- 8) Freeze in continuous freezer to desired overrun (100% recommended)
- 9) Harden in tunnel at -40°C
- 10) Store at a temperature below -25°C

The use of the described pectin/calcium system in the recipe will help reduce the ice crystal formation in the ice cream during production and during freeze storage of the ice cream.

The ice-cream has thus a good heat-shock resistance, and a dry and creamy mouthfeel, with clean-eating properties.

The meltdown of the ice cream made according to the formulation is comparable to an ice cream made with xanthan gum (0.18%). Both of them have a relatively slow meltdown, and slower meltdown compared to standard commercial ice cream.

Also the "first drop" (minutes before the ice cream starts to drip under standardised conditions) is very similar for the ice cream made with pectin and xanthan gum.

SUBSTITUTE SHEET (RULE 26)

Increasing the pectin dosage to only 0.30 % gives a considerably improvement of these described properties.

5 TABLE VII

Ingredients	wt %
Dairy cream, 38% milk fat	23.65
Skimmed milk	53.55
Skimmed milk powder	4.90
Pectin composition according to invention	0.25*
Sugar	12.00
Glucose syrup, DE 42, 75% TS	5.35
CREMODAN® Super	0.3
Flavouring	Optional
Colouring	Optional
Total	100.00
Fat ( per cent)	9.00
MSNF (per cent)	10.80
Total solids (per cent)	36.35

\* corresponding to a calcium sensitive pectin population content of 0.15 wt%

10

# PROTOCOL I

## CALCIUM SENSITIVITY INDEX (CF)

15

Calcium sensitivity is measured as the viscosity of a pectin dissolved in a solution with 57.6 mg calcium/g pectin divided by the viscosity of exactly the same amount of pectin in solution, but without added calcium. A calcium insensitive pectin has a CF value of

SUBSTITUTE SHEET (RULE 26)

1.

4.2 g pectin sample is dissolved in 550 ml hot water with efficient stirring. The solution is cooled to about 20°C and the pH adjusted to 1.5 with 1N HCl. The pectin solution is adjusted to 700 ml with water and stirred. 290 g of this solution is measured individually into 2 viscosity glasses. 10 ml water is added to one of the glasses (double determinations) and 10 ml of a 250 mM CaCl<sub>2</sub> solution is added to the other glass under stirring.

50 ml of an acetate buffer (0.5 M, pH about 4.6) is added to both viscosity glasses under efficient magnetic stirring, thereby bringing the pH of the pectin solution up over pH 4.0. The magnets are removed and the glasses left overnight at 20°C. The viscosities are measured the next day with a Brookfield viscometer. The calcium sensitivity index is calculated as follows:

$$CF = \frac{\text{Viscosity of a solution with } 57.6 \text{ mg Ca}^{2+} / \text{g pectin}}{\text{Viscosity of a solution with } 0.0 \text{ mg Ca}^{2+} / \text{g pectin}}$$

## PROTOCOL II

### YIELD STRESS

An aqueous composition was prepared in accordance with the following procedure

- 1) The pectin composition was blended in hot water (80-90°C) while agitating vigorously.
- 2) Diluting concentrated strawberry juice\* at a ratio of 1:6.5 with water to provide strawberry juice having a solid content of 10.0% and a calcium content of 200 ppm.
- 3) Mix the diluted strawberry juice and pectin composition while agitating vigorously
- 4) Heat to boiling point
- 5) Add calcium lactate.5H<sub>2</sub>O to provide a level of Ca<sup>2+</sup>/pectin of 150mg/g\*\*
- 6) Adjust pH to 3.8-4.0
- 7) Adjust soluble solid content to 30%

SUBSTITUTE SHEET (RULE 26)

8) Cool to 30°C under agitation to provide an aqueous composition

\* available from Dinter GmbH.

Specification soluble solid content of 65 %

5 calcium content of approximately 1300ppm  
produced by concentration of strawberry juice, obtained from  
pressing fresh/frozen strawberries  
Brix: 64.0-66.0  
Acidity: 6.0-7.8% as citric acid, pH 8.1

10

\*\* This amount of calcium lactate has been optimised for high ester pectin. The  
amount of calcium lactate may be varied if a medium ester or low ester pectin is to  
be considered. This optimisation may be achieved by the performance of a series of  
simple experiments varying the amount of calcium lactate to optimise the high yield  
15 stress/non-gelling properties of an aqueous composition containing a pectin  
composition of the present invention.

The rheological effect of the strawberry juice concentrate is directly related to the  
amount of calcium in the juice concentrate. The concentrate may be analysed and  
20 replaced with a calcium solution e.g. a calcium lactate solution. The present  
Protocol has been repeated with such a solution and identical results were obtained.

The aqueous composition was stored at room temperature for minimum 48 hours and  
applied to a Stress Tech CS rheometer supplied by Reologica Instruments AB. The  
25 pectin sample was applied as gently as possible without stirring of the sample.

The rheometer was configured as follows

Programme:	Yield Stress
Measurement System:	CC 25 (25mm concentric cylinders)
30 Equilibrium time:	900 s
Stress:	0.1429 - 10.00 Pa, up
Measurement time:	300.0 s in 60 intervals

SUBSTITUTE SHEET (RULE 26)

Temperature: 25<sup>27</sup>°C

5 The procedure is repeated, if necessary, until the ratio of pectin composition to water required to achieve a maximum viscosity of approximately 500 Pa is determined. This ratio provides a standardised aqueous composition.

10 The yield stress of a standardised pectin composition is then determined by applying (as gently as possible without stirring of the sample) the standardised aqueous composition which has been stored at room temperature for minimum 48 hours to a Stress Tech CS rheometer supplied by Reologica Instruments AB.

The rheometer was configured as follows

15	Programme:	Yield Stress
	Measurement System:	CC 25 (25mm concentric cylinders)
	Equilibrium time:	900 s
	Stress:	0.1429 - 10.00 Pa, up
	Measurement time:	300.0 s in 60 intervals
	Temperature:	25°C

20

The yield stress is determined as the stress giving the maximum viscosity during stress viscometry.

### PROTOCOL III

25

### NON-GELLING

An aqueous composition was prepared in accordance with the following procedure

- 30
- 1) The pectin composition was blended in hot water (80-90°C) while agitating vigorously.
  - 2) Diluting concentrated strawberry juice\* at a ratio of 1:6.5 with water to provide strawberry juice having a solid content of 10.0% and a calcium content of 200 ppm.

SUBSTITUTE SHEET (RULE 26)

28

- 3) Mix the diluted strawberry juice and pectin composition while agitating vigorously
- 4) Heat to boiling point
- 5) Add calcium lactate.5H<sub>2</sub>O to provide a level of Ca<sup>2+</sup>/pectin of 150mg/g\*\*
- 6) Adjust pH to 3.8-4.0
- 7) Adjust soluble solid content to 30%
- 8) Cool to 30°C under agitation to provide an aqueous composition

\* available from Dinter GmbH.

Specification soluble solid content of 65 %

calcium content of approximately 1300ppm

obtained from pressing fresh/frozen strawberries

Brix: 64.0-66.0

Acidity: 6.0-7.8% as citric acid, pH 8.1

\*\* This amount of calcium lactate has been optimised for high ester pectin. The amount of calcium lactate may be varied if a medium ester or low ester pectin is to be considered. This optimisation may be achieved by the performance of a series of simple experiments varying the amount of calcium lactate to optimise the high yield stress/non-gelling properties of an aqueous composition containing a pectin composition of the present invention.

The rheological effect of the strawberry juice concentrate is directly related to the amount of calcium in the juice concentrate. The concentrate may be analysed and replaced with a calcium solution e.g. a calcium lactate solution. The present Protocol has been repeated with such a solution and identical results were obtained.

The aqueous composition was stored at room temperature for minimum 48 hours and applied to a Stress Tech CS rheometer supplied by Reologica Instruments AB. The pectin sample was applied as gently as possible without stirring of the sample.

The rheometer was configured as follows

SUBSTITUTE SHEET (RULE 26)

29

Programme: Yield Stress  
Measurement System: CC 25 (25mm concentric cylinders)  
Equilibrium time: 900 s  
Stress: 0.1429 - 10.00 Pa, up  
5 Measurement time: 300.0 s in 60 intervals  
Temperature: 25°C

10 The procedure is repeated, if necessary, until the ratio of pectin composition to water required to achieve a yield stress of approximately 1 Pa is determined. This ratio provides a standardised aqueous composition.

The yield stress of a standardised pectin composition is then determined by applying (as gently as possible without stirring of the sample) the standardised aqueous composition which has been stored at room temperature for minimum 48 hours to a  
15 Stress Tech CS rheometer supplied by Reologica Instruments AB.

The rheometer was configured as follows

20 Programme: Yield Stress  
Measurement System: CC 25 (25mm concentric cylinders)  
Equilibrium time: 900 s  
Stress: 0.1429 - 10.00 Pa, up  
Measurement time: 300.0 s in 60 intervals  
Temperature: 25°C

25

The presence of gelation is determined by measuring the maximum viscosity during stress viscometry.

#### PROTOCOL IV

30

#### FLOW CURVE

To evaluate shear reversibility an aqueous composition as described in protocol II

SUBSTITUTE SHEET (RULE 26)

30

was prepared and a flow curve was obtained in accordance with the following.

The sample was stored at room temperature for minimum 48 hours and applied to a Bohlin VOR rheometer. The sample was applied as gently as possible without stirring of the sample.

The rheometer was configured as follows

Programme:	Viscometry
Measurement System:	CC 25 (25mm concentric cylinders)
Torque bar:	20.25 gcm or 41.10 gcm
Shear rate:	0.06 - 92 s <sup>-1</sup> , up and down
Equilibrium time:	900 s
Measurement time:	608 s in 33 intervals
Temperature:	25°C

The viscosity and shear stress as a function of shear rate is followed.

The viscosity profile as a function of shear rate illustrates the degree of shear thinning of the system.

The viscosity at any given shear rate, in particular 0.1s<sup>-1</sup> may be readily determined.

The area between the "up" and "down" curves in the flow curve corresponds to the degree of thixotropy.

#### PROTOCOL V

#### DEGREE OF ESTERIFICATION (%DE)

To 50 ml of a 60 % isopropanol and a 5 % HCl solution is added 2.5 g pectin sample and stirred for 10 min. The pectin solution is filtered through a glass filter and washed with 15 ml 60 % isopropanol/5 % HCl solution 6 times followed by further washes with 60% isopropanol until the filtrate is free of chlorides. The filtrate is dried

SUBSTITUTE SHEET (RULE 26)



overnight at 80°C.

20.0 ml 0.5 N NaOH and 20.0 ml 0.5 N HCl is combined in a conical flask and 2 drops of phenolphthalein is added. This is titrated with 0.1 N NaOH until a permanent colour change is obtained. The 0.5 N HCl should be slightly stronger than the 0.5N NaOH. The added volume of 0.1 N NaOH is noted as  $V_0$ .

0.5 g of the dried pectin sample (the filtrate) is measured into a conical flask and the sample is moistened with 96% ethanol. 100 ml of recently boiled and cooled distilled water is added and the resulting solution stirred until the pectin is completely dissolved. Then 5 drops of phenolphthalein are added and the solution titrated with 0.1 N NaOH (until a change in colour and pH is 8.5). The amount of 0.1 N NaOH used here is noted as  $V_1$ . 20.0 ml of 0.5 N NaOH is added and the flask shaken vigorously, and then allowed to stand for 15 min. 20.0 ml of 0.5 N HCl is added and the flask is shaken until the pink colour disappears. 3 drops of phenolphthalein are then added and then the resultant solution is titrated with 0.1 N NaOH. The volume 0.1 N NaOH used is noted as  $V_2$ .

The degree of esterification (% DE: % of total carboxy groups) is calculated as follows:

$$\% \text{ DE} = \frac{V_2 - V_0}{V_1 + (V_2 - V_0)}$$

#### 25 PROTOCOL VI

#### METHOD OF DETERMINING CSP CONTENT

A pectin composition was fractionated into a calcium sensitive fraction (CSP) and a none calcium sensitive fraction (NCSP) in accordance with the method below

- 30
- Dissolve 1 % sugar free pectin in water
  - Adjust pH to 4.5 with a solution of 10 %  $\text{Na}_2\text{CO}_3$
  - Make a fractionation solution of 60 mM  $\text{CaCl}_2$ /16% IPA/water
  - Measure 20 ml fractionation solution in a 80 ml centrifugation glass

SUBSTITUTE SHEET (RULE 26)

32

- Inject about 20 grams pectin solution into the fractionation solution, the precise amount to be noted
- Centrifuge at 5000 rpm for 20 minutes
- Separate
- 5 - Add 30 ml fractionation solution, diluted in a ratio of 1:1 with water
- Mix the sedimented gel and the diluted fractionation solution with a spatula and centrifuge at 5000 rpm for another 5 minutes
- Repeat this procedure twice
- Dissolve the gel with a few drops of 3 N HCl and stir with a spatula
- 10 - Add 60 ml 60 % IPA/3% HCl/37% H<sub>2</sub>O to the dissolved gel. Mix and centrifuge at 5000 rpm for 5 minutes
- Wash out chloride from the material with 60 ml 60 % IPA four times. Each wash is mixed, centrifuged and separated
- The CSP fraction is transferred to a low weight plastic petri dish (which has been
- 15 weighed on beforehand)
- Dry at 40 °C over night

#### PROTOCOL VII

#### REDUCED VISCOSITY

20

A quantity of pectin corresponding to 90 mg pectin dry matter is weighted into a 150ml Erlenmeyer flask together with 100 g buffer solution which is prepared by dissolving 1 g sodium hexametaphosphate in distilled water and adjusting the pH value to 4.5 with a few drops of 4N HCL. After a few hours of stirring the pectin is

25 dissolved. If it is not clear the solution is filtered through a porosity 2-filter crucible. Dropping periods of the resulting pectin solution and the buffer solution, respectively are measured at 20°C in a "falling ball viscometer" (Hoeppler viscometer)

The specific viscosity is:

30

$$\eta_{sp} = (\text{Dropping period for pectin solution} / \text{dropping period for buffer solution}) - 1$$

SUBSTITUTE SHEET (RULE 26)

The reduced viscosity is:

$$\eta_{\text{red}} = \eta_{\text{sp}}/c = \eta_{\text{sp}}/1 \text{ (L/g)}$$

5 where c is the concentration of pectin powder with 10 % water, expressed in g/L.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system  
10 of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention  
15 which are obvious to those skilled in chemistry or related fields are intended to be within the scope of the following claims.

SUBSTITUTE SHEET (RULE 26)

CLAIMS

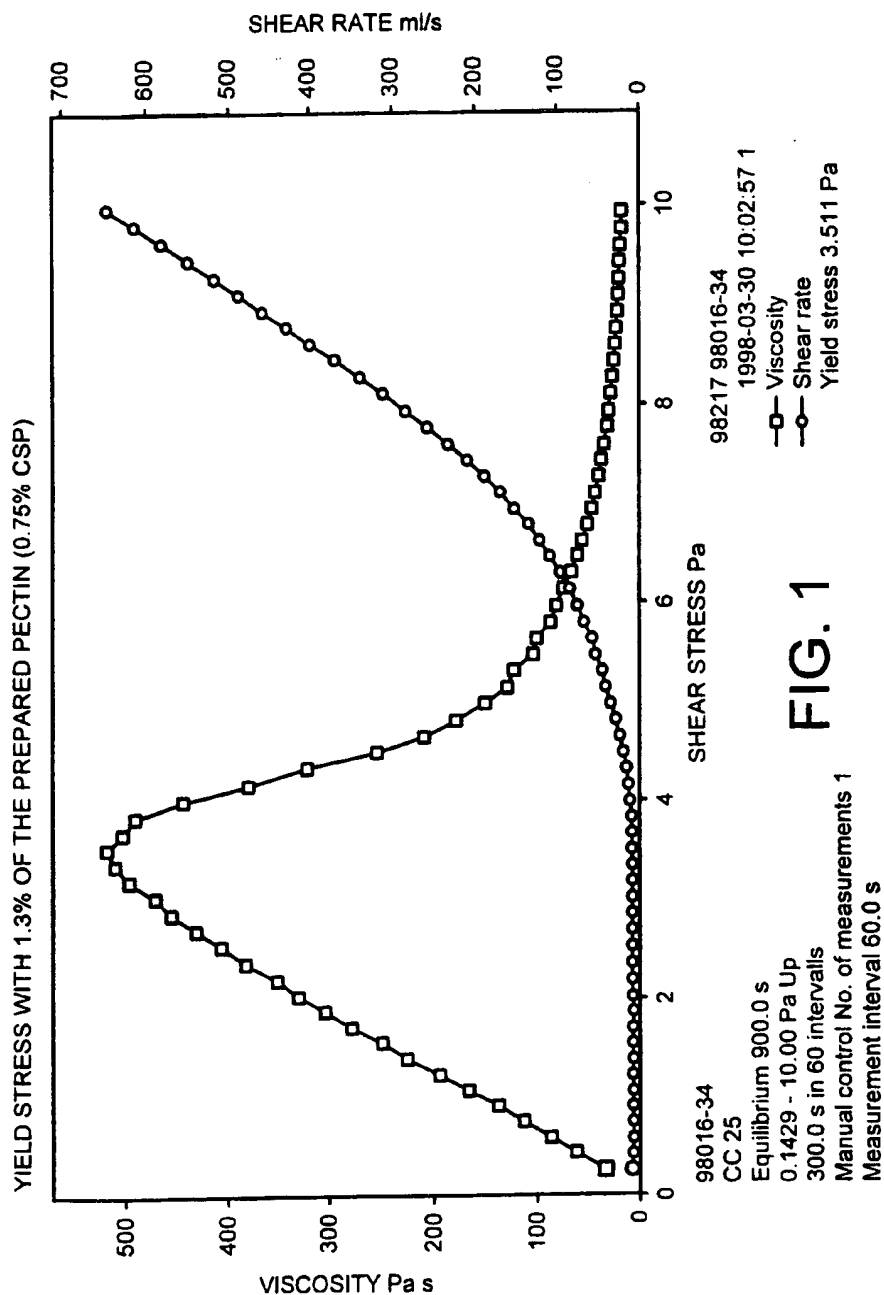
1. Use of a pectin composition to prepare an aqueous composition having high yield stress characteristics without substantial gelation, wherein the pectin composition comprises at least a population of pectin that is calcium sensitive; wherein the pectin composition is capable of imparting high yield stress characteristics to an aqueous composition without causing substantial gelation of the aqueous composition.
2. Use according to claim 1 wherein the pectin composition comprises no greater than 5.0 wt% of the aqueous composition based on the total weight of the aqueous composition.
3. Use according to claim 2 wherein the pectin composition comprises no greater than 0.1-1.5 wt% of the aqueous composition based on the total weight of the aqueous composition.
4. Use according to any one of claims 1, 2 or 3 wherein the aqueous composition comprises  $\text{Ca}^{2+}$  ions.
5. Use according to claim 4 wherein the aqueous composition comprises 25-300 mg of  $\text{Ca}^{2+}$  ions per gram of pectin composition.
6. Use according to claim 4 wherein the aqueous composition comprises 50-150 mg of  $\text{Ca}^{2+}$  ions per gram of pectin composition.
7. Use according to any one of the preceding claims wherein the aqueous composition has a low viscosity at a shear rate of  $0.1 \text{ s}^{-1}$ .
8. An aqueous composition prepared in accordance with any one of the preceding claims.
9. Use as substantially described herein with reference to any one of the Examples.

SUBSTITUTE SHEET (RULE 26)

10. An aqueous composition as substantially described herein with reference to any one of the Examples.

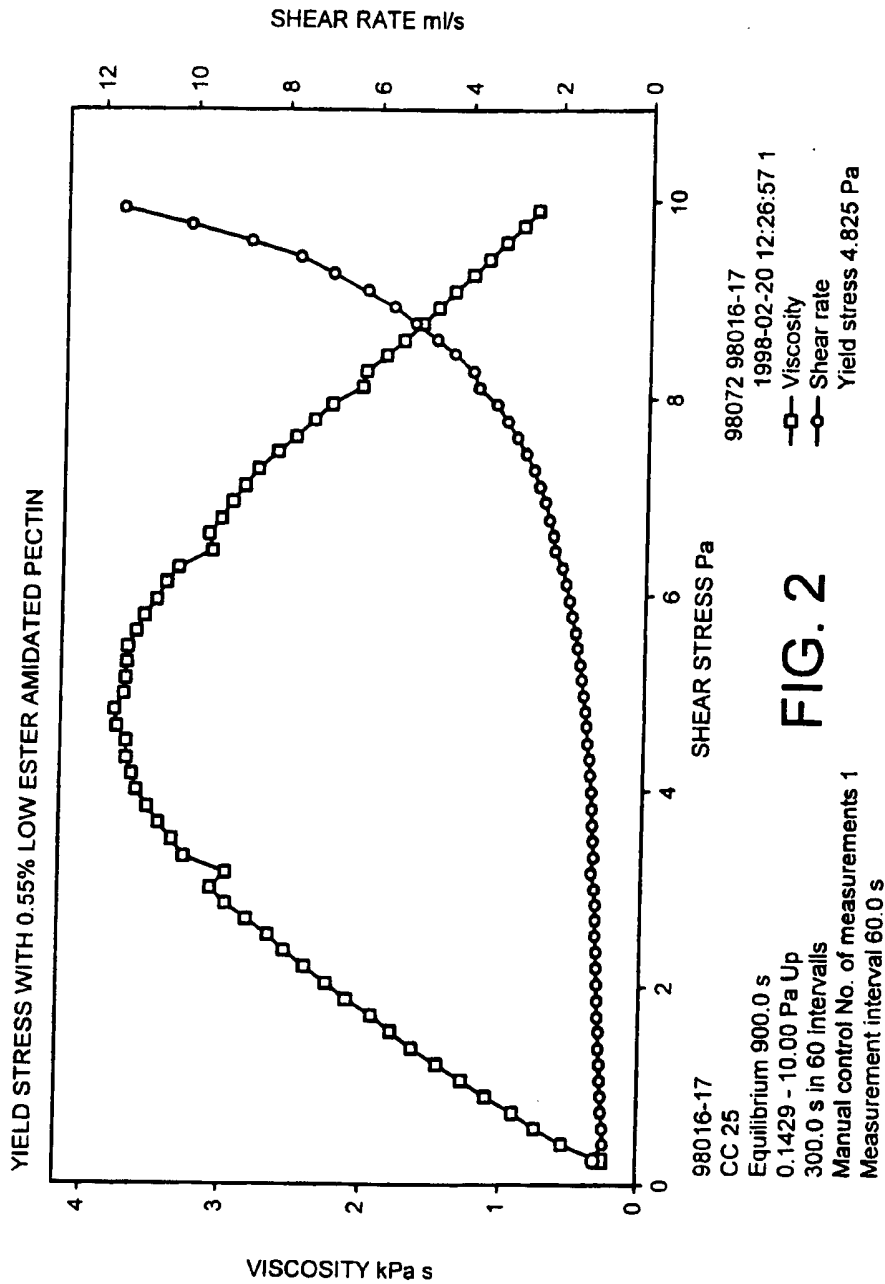
SUBSTITUTE SHEET (RULE 26)

1/7



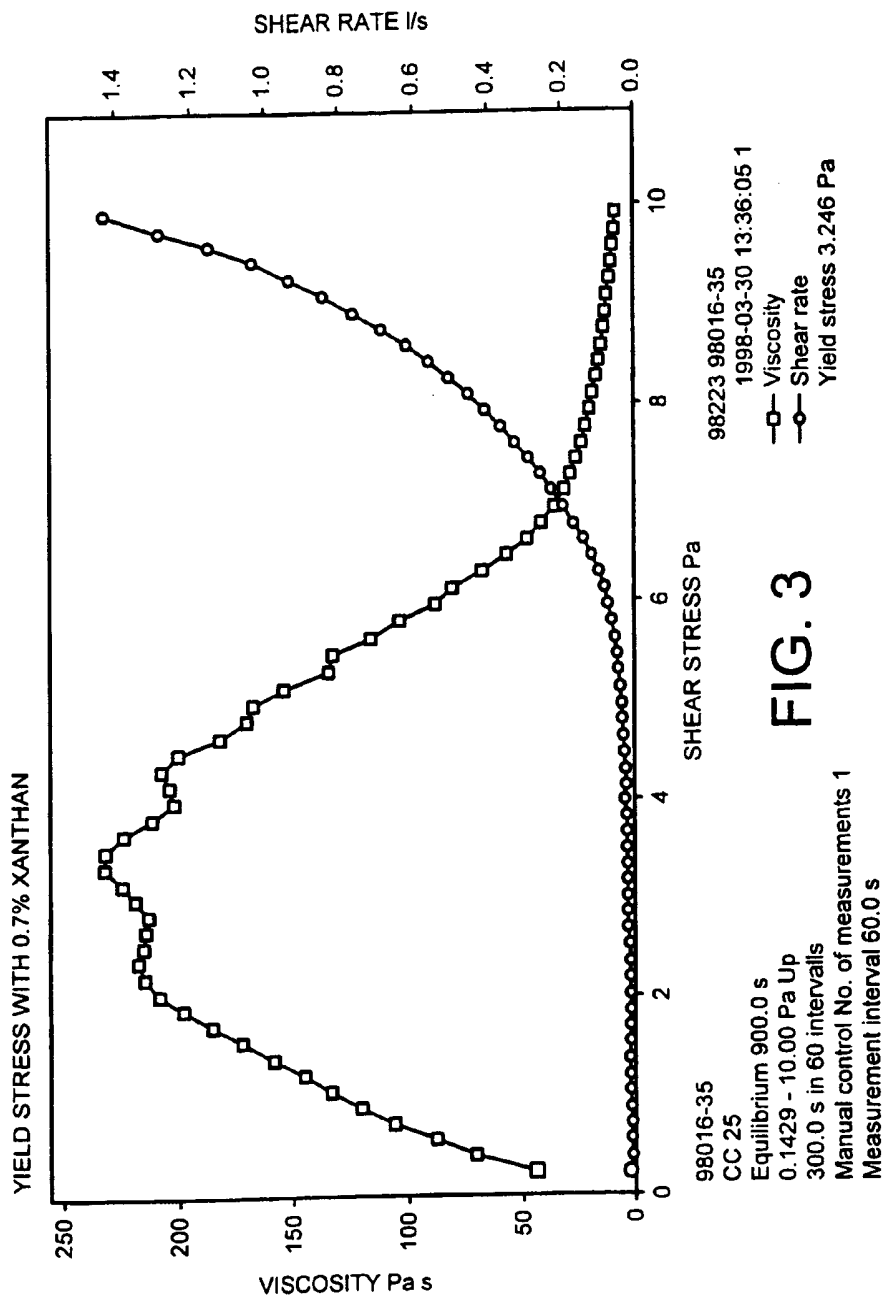
SUBSTITUTE SHEET (RULE 26)

2/7



SUBSTITUTE SHEET (RULE 26)

3/7



SUBSTITUTE SHEET (RULE 26)



4/7

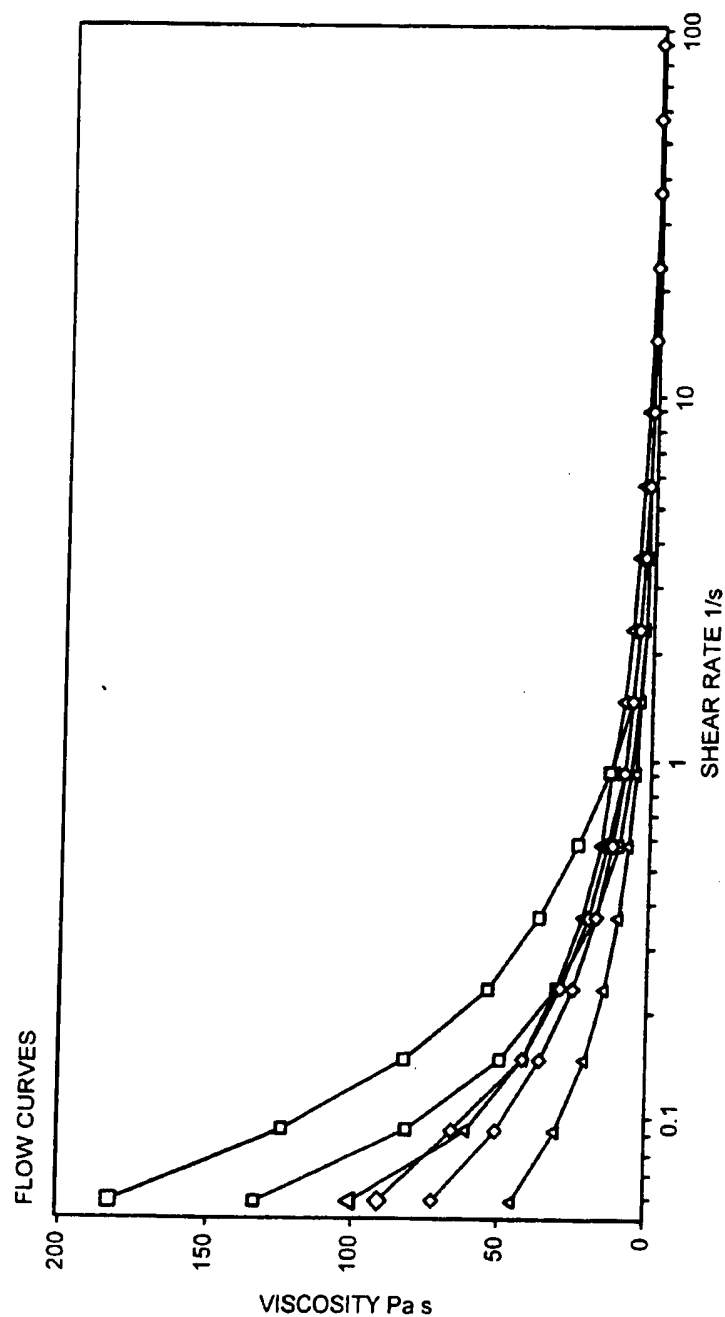


FIG. 4

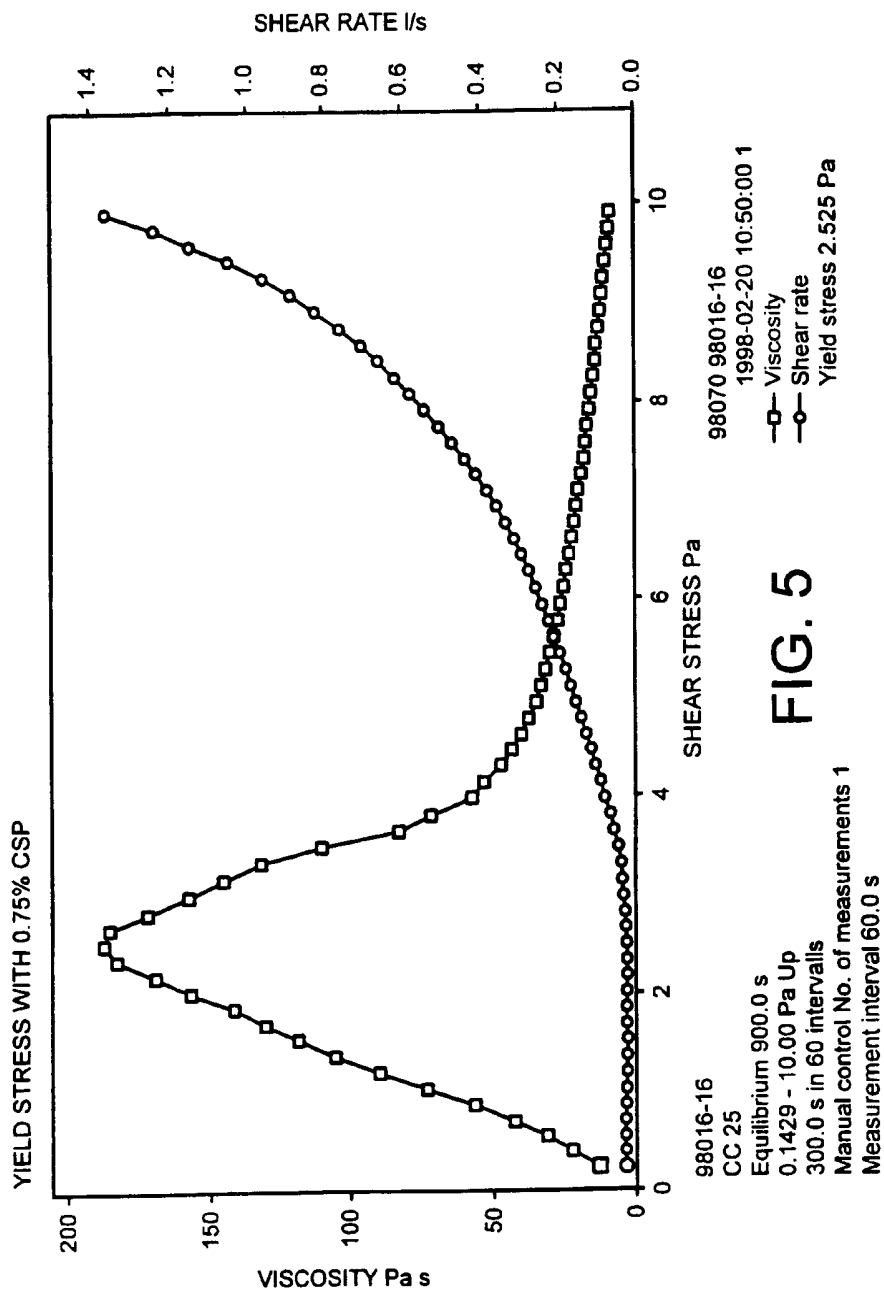
998141 0,55% low ester amidated pectin  
1998-02-24 13:18:41 1  
—□— Viscosity

98280 1,3% of the prepared pectin (0,75% CSP)  
1998-03-31 08:29:41 0  
—△— Viscosity

98282 0,7% xanthan  
—◇— Viscosity

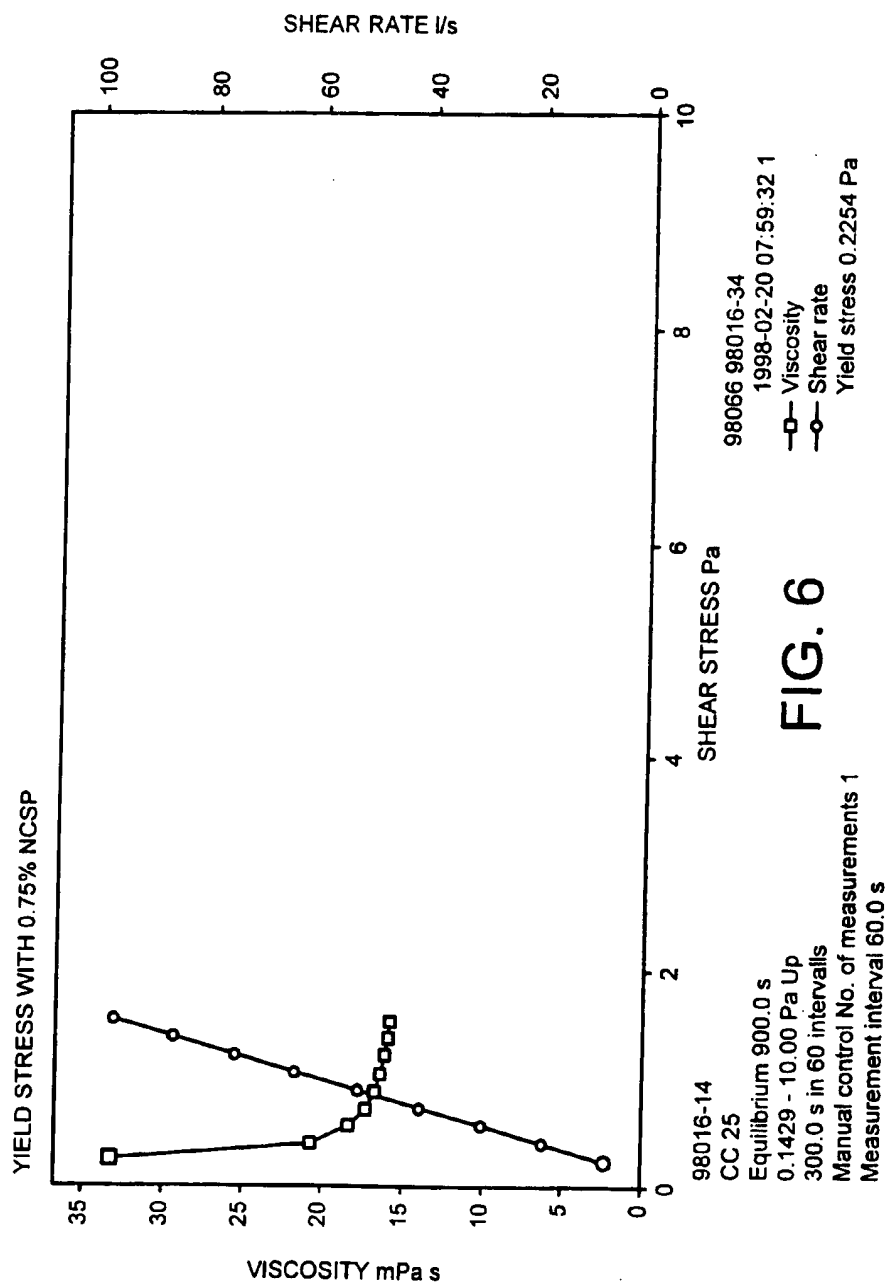
SUBSTITUTE SHEET (RULE 26)

517



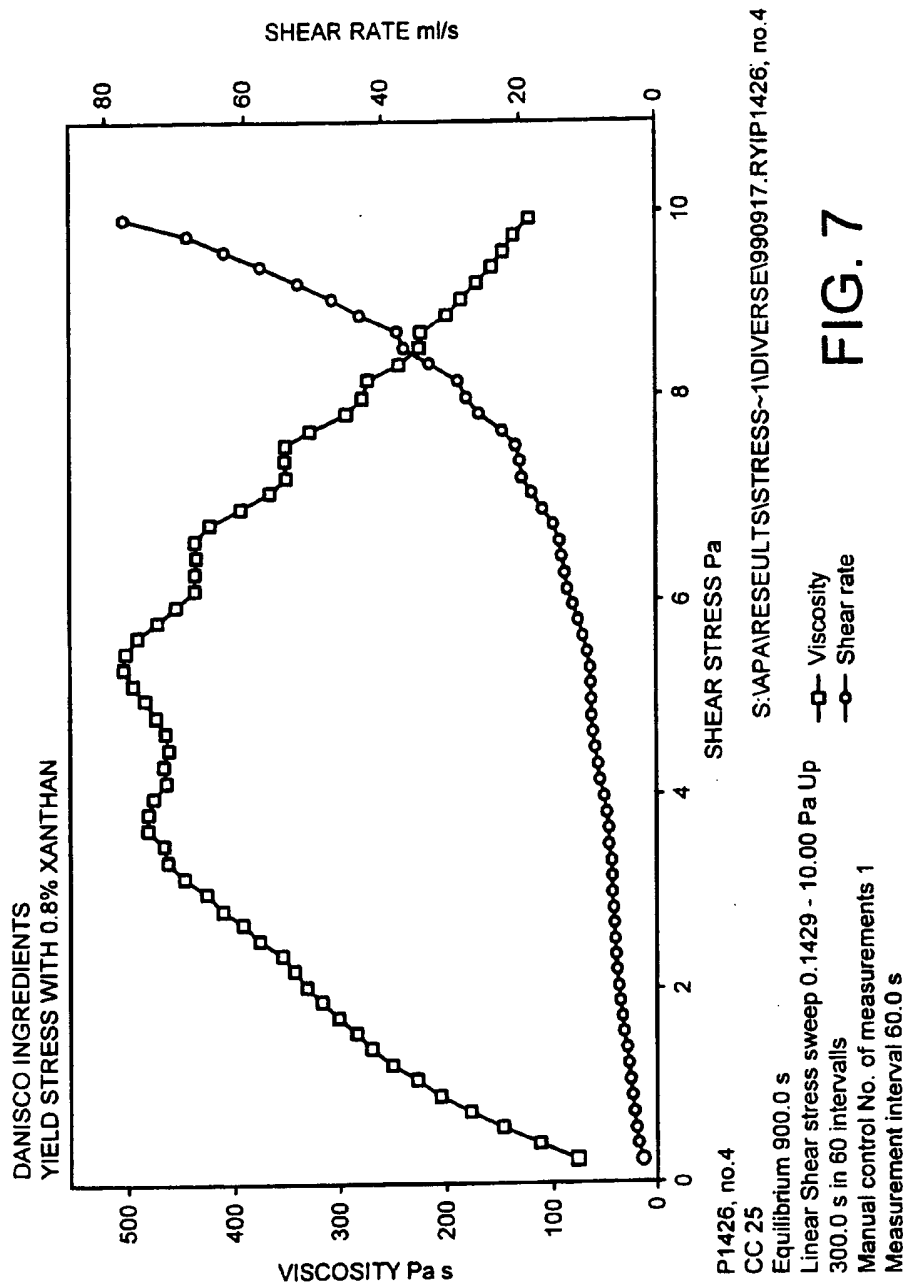
**SUBSTITUTE SHEET (RULE 26)**

6/7



SUBSTITUTE SHEET (RULE 26)

7/7



SUBSTITUTE SHEET (RULE 26)

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 99/01476

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23L1/0524 A23L1/064 A23L2/52 A23C9/123 A23L1/24  
A23G9/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 664 300 A (HERCULES INC) 26 July 1995 (1995-07-26) page 3, line 9 - line 11 page 3, line 22 - line 37 page 5, line 52 - page 6, line 22 examples 2-17 ---	1-10
X	GB 2 194 876 A (NYIRSEG KONZERVIPARI VALLALAT) 23 March 1988 (1988-03-23) page 1, line 31 - line 57 example 2 claims 1-4 --- -/--	1-10



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

16 November 1999

Date of mailing of the international search report

06. 12. 1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Vuillamy, V

Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 2

## INTERNATIONAL SEARCH REPORT

Interno 31 Application No

PCT/IB 99/01476

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 03574 A (DANISCO ;CHRISTENSEN TOVE MARTEL IDA EL (DK); KREIBERG JETTE DINA) 6 February 1997 (1997-02-06) cited in the application page 57 example 1 ---	1-10
P,X	WO 98 47391 A (KREIBERG JETTE DINA ;DANISCO (DK); HYTTEL SUSANNE (DK); CHRISTENSE) 29 October 1998 (1998-10-29) cited in the application page 27 page 42 -page 43; tables I,II,III ---	1-10
X	DATABASE WPI Section Ch, Week 198021 Derwent Publications Ltd., London, GB; Class D13, AN 1980-37457C XP002122724 & JP 55 050885 A (AJINOMOTO KK), 14 April 1980 (1980-04-14) abstract ---	1-5,7-10
X	EP 0 709 033 A (YAKULT HONSHA KK) 1 May 1996 (1996-05-01) page 1, line 56 -page 4, line 31 page 4, line 45 -page 5, line 46 page 7, line 49 - line 55 page 8, line 11 - line 18 examples 4-7 ---	1-10
X	WO 89 12648 A (KOEENHAVNS PEKTINFABRIK AS) 28 December 1989 (1989-12-28) page 1, line 34 -page 2, line 10 -----	1-10

# INTERNATIONAL SEARCH REPORT

Inte. tional application No.  
PCT/IB 99/01476

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box 1.2

Present claims 1-10 relate to pectin based compositions (and their use) defined by reference to a desirable characteristic or property, namely a 'high yield stress characteristics without substantial gelation'. Claim 7 refers to a 'low viscosity at a shear rate of 0.1'.

The present description (cf. page 4, par.2) further mentions that this result is achieved by:

- control on the degree of esterification
- proportion of the pectin composition which is calcium sensitive
- calcium sensitivity of the calcium sensitive population of pectin.
- molecular weight of the pectin
- concentration of the pectin.

The claims cover all products and use having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products and use. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the products and use by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely (cf. description page 3, par.3-4; page 5, par.1-3; page 9, par.5-6) for high ester pectin (DE of 50-90%) having a calcium sensitive index CF > 1.2 .

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 99/01476

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0664300 A	26-07-1995	BR 9404806 A	01-08-1995
		CN 1111261 A	08-11-1995
		CZ 9403023 A	14-06-1995
		JP 7196706 A	01-08-1995
		PL 306079 A	12-06-1995
GB 2194876 A	23-03-1988	BE 905447 A	16-01-1987
		DE 3630009 A	10-03-1988
		NL 8602223 A	05-04-1988
WO 9703574 A	06-02-1997	AU 6614496 A	18-02-1997
		BR 9609791 A	16-03-1999
		CA 2225481 A	06-02-1997
		CN 1195970 A	14-10-1998
		CZ 9800117 A	17-06-1998
		EP 0839006 A	06-05-1998
		JP 11509102 T	17-08-1999
		NZ 313625 A	30-08-1999
		PL 324503 A	25-05-1998
WO 9847391 A	29-10-1998	AU 6932498 A	13-11-1998
JP 55050885 A	14-04-1980	JP 60029465 B	10-07-1985
EP 0709033 A	01-05-1996	JP 8112058 A	07-05-1996
		AU 693931 B	09-07-1998
		AU 3432595 A	02-05-1996
		US 5690975 A	25-11-1997
WO 8912648 A	28-12-1989	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)

